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Prospective Comparison of Cell Cultures and Nucleic Acid Amplification Tests for Laboratory Diagnosis of *Chlamydia trachomatis* Infections

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Specimens submitted in M5 medium for cell culture detection of *Chlamydia trachomatis* were tested by nucleic acid amplification testing (NAAT) and in cell cultures. Of 35 (genital) and 26 (nongenital) specimens positive for *C. trachomatis*, 21 and 14 specimens, respectively, were detected exclusively by NAAT. NAAT is significantly ($P < 0.0001$) more sensitive than cell culture and should be considered the new "gold standard" for the laboratory diagnosis of *C. trachomatis* infections.

Chlamydia trachomatis is the most common cause of reportable cases of sexually transmitted infections (7, 16). Sensitive, Food and Drug Administration (FDA)-approved nucleic acid amplification testing (NAAT) has replaced poorly standardized cell culture methods for the laboratory diagnosis of these infections (5, 13, 15, 17).

Specimens submitted to our laboratory with a specific request for *C. trachomatis* culture in M5 medium (Remel, Lenexa, KS) were inoculated into cell cultures and simultaneously processed by NAAT for both *C. trachomatis* and *Neisseria gonorrhoeae* (BDProbeTec ET). The goal of our study was to assess the performance characteristics of NAAT as a possible exclusive replacement for cell cultures for the testing of all specimens submitted for the laboratory diagnosis of *C. trachomatis* infections.

Shell vials containing cycloheximide-treated McCoy cells (Diagnostic Hybrids Inc., Athens, OH) on coverslips were inoculated with 0.1 ml of the specimen extract in M5 transport medium and centrifuged at 700 × *g* for 60 min. Cell cultures were incubated at 35°C for 48 h, stained with fluorescein-conjugated monoclonal antibody specific for *C. trachomatis* (Trinity Biotech Co., Wicklow, Ireland), and examined at 200 × for the detection of *C. trachomatis*-infected cells (18).

For NAAT, specimens (200 μl) in M5 medium were placed into BD diluent, lysed by heating at 114°C for 30 min, and assayed by the BDProbeTec ET system using the BD Viper (BD Biosciences, Sparks, MD) for sample processing and detection of *C. trachomatis* and *N. gonorrhoeae* following the manufacturer's instructions.

Of 383 genital specimens (cervix, 179; vagina, 166; urethra, 38) submitted to Mayo Clinic for cell culture diagnosis of *C. trachomatis* infections processed also by NAAT, 14 (3.7%) were positive for the organism by both methods (Table 1). Two specimens (cervix) were toxic in cell culture, and diagnostic results were not available by that method. In addition, 21 specimens were exclusively positive for *C. trachomatis* by NAAT (150% increase in the rate of detection compared with that of cell cultures). These 21 discrepant results were confirmed as positive by direct fluorescent antibody to specifically detect the elementary bodies of *C. trachomatis* (Tables 1 and 2). Importantly, there were no specimens that were detected by culture that were not detected by NAAT (100% specificity) (Table 1). Of the 35 (14 plus 21) NAAT-positive *C. trachomatis* specimens, 5 (cervix, 4; vagina, 1) (14.3%) were also positive for both *C. trachomatis* and *N. gonorrhoeae* (Table 2). In addition, 6 (cervix, 5; vagina, 1) other specimens of the 348 *C. trachomatis*-negative specimens (383 minus 35) (1.7%) tested were positive for *N. gonorrhoeae* but negative for *C. trachomatis* (Table 2).

We also compared NAAT and cell culture for the detection of both *C. trachomatis* and *N. gonorrhoeae* for specimens obtained from other than genital sites. Of 337 nongenital specimens (eye, 263; nares, 38; throat, 36), 12 (3.6%) were positive for *C. trachomatis* by both NAAT and cell cultures (Table 1). Similar to results obtained with genital tract specimens, *C. trachomatis* was detected exclusively by NAAT in 14 specimens (117% increase in the rate of detection compared with cell cultures) (Table 1). Of these 14 specimens, the presence of *C. trachomatis* was confirmed by immunofluorescence in 12 of the samples that had adequate specimen volumes (Table 2). Consistent with our results with genital tract specimens, *N. gonorrhoeae* was detected as a coinfection with *C. trachomatis* in four specimens (eye, 3; throat, 1) (Table 2).

Our data clearly indicate the superiority of NAAT compared to cell culture for the laboratory diagnosis of *C. trachomatis* infections with both genital and nongenital specimen sources. Discrepant results (NAAT positive and cell cultures negative) were confirmed by immunofluorescence. Although NAAT testing for *N. gonorrhoeae* may cross-react with *Neisseria cinerea* and *Neisseria lactamica*, similar homologies of target DNA have not been noted in kit package inserts for *C. trachomatis* with other microorganisms (1). Further, strains of bacteria other than *C. trachomatis* may cross-react in the immunofluorescence test; however, the elementary bodies of this organism can be distinguished from other bacteria by characteristic morphology. Therefore, based on the consistent results obtained

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TABLE 1. Comparison of cell culture and BDProbeTec ET (NAAT) for laboratory diagnosis of *Chlamydia trachomatis* infection

Result by NAAT	No. of specimens tested by cell culture[a]			
	Genital		Nongenital	
	Positive	Negative	Positive	Negative
Positive	14	21*	12	14*
Negative	0	348	0	311

[a] *, $P < 0.0001$.

with specimens from both genital and nongenital sites together with morphological criteria (immunofluorescence test), the discrepant NAAT tests should be considered confirmatory for *C. trachomatis*.

Our results support and confirm the superiority of NAAT testing to cell culture by using an extended "gold standard" (two or more tests positive with nonculture methods) for discrepant analysis of results (2–4, 6, 8, 10, 12, 16, 17). Recent data from an experimental protocol indicate that two NAAT platforms (APTIMA Combo 2; BDProbeTec ET) were equal or superior to cell cultures cultivations for the detection of *C. trachomatis* infections with rectal specimens obtained from men who have sex with men (J. Schachter, J. V. Mancada, S. Liska, and J. D. Klausenet, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. L-1006, 2004).

FDA-approved NAAT with commercial platforms also provides the ability for detecting *N. gonorrhoeae* from patients (1, 11). Overall, 11 of 383 (2.9%) genital specimens were positive for *N. gonorrhoeae* and 5 of 11 (45%) were coinfections (cervix, 4; vagina, 1) with *C. trachomatis*. As outlined earlier, there were six instances (cervix, 5; vagina, 1) where *N. gonorrhoeae* was exclusively detected by the NAAT method. Infections with *N. gonorrhoeae* are significant because optimal antibacterial therapy differs for the treatment of this infection and for *C. trachomatis* (19).

TABLE 2. Sources and test methods used to detect *C. trachomatis* and *N. gonorrhoeae* from 720 specimens submitted for culture for *C. trachomatis* infections

Source (no. of specimens)	Total no. of specimens positive for *C. trachomatis*	No. of specimens positive for *C. trachomatis* and/or *N. gonorrhoeae*	Test result[a]			
			C. trachomatis			*N. gonorrhoeae* NAAT
			Culture	NAAT	DFA	
Genital (383)						
Cervix (179)	24	10	+	+	ND[b]	−
		7	−	+	+	−
		4	−	+	+	+
		1	−	+	+	−
		2	Toxic	+	+	−
Vagina (166)	7	5	−	+	+	−
		1	+	+	ND	−
		1	−	+	+	+
Urethra (38)	4	3	+	+	ND	−
		1	−	+	+	−
Total[d]	35	35				
Nongenital (337)						
Eye (263)	16	8	+	+	ND[b]	−
		7	−	+	+[c]	−
		1	−	+	+	+
		2	−	−	ND	+
Nasal (38)	7	4	+	+	ND	−
		3	−	+	+	−
Throat (36)	3	3	−	+	+	−
		1	−	−	ND	+
Total[d]	26	29				

[a] +, positive; −, negative.
[b] DFA was not done (ND) because *C. trachomatis* was recovered by cell cultures.
[c] Two specimens were not confirmed as positive due to limited volume of the original extract.
[d] A total of 35 genital specimens tested positive for *C. trachomatis*, but the number of specimens positive by any given test varied as follows: by culture, 14 specimens; by NAAT, 35 specimens; and by DFA, 21 specimens. A total of 26 nongenital specimens tested positive for *C. trachomatis*, but the number of specimens positive by any given test varied as follows: by culture, 12 specimens; by NAAT, 26 specimens; and by DFA, 14 specimens. A total of five genital and four nongenital specimens tested positive by NAAT for *N. gonorrhoeae.*

Cell culture detection of *C. trachomatis* is sometimes desirable for additional laboratory characterization of the organisms in the NAAT is positive. For this purpose, an aliquot of the original specimen submitted in M5 medium, and temporarily stored at 4°C, can be retrospectively inoculated into cell cultures in an attempt to recover the organism.

Expenses related to test performance in our institution has indicated that NAAT testing is about 75% less costly than cell culture methods for the laboratory diagnosis of *C. trachomatis* infections. In addition, the cost of NAAT for both *C. trachomatis* and *N. gonorrhoeae* is about equivalent to the cell culture fee for cultivation which yields results only for *C. trachomatis*.

The results of our study that included a wide variety of specimen types demonstrated that NAAT with immunofluorescence confirmation of discrepant results (NAAT positive and cell cultures negative) was more sensitive (genital, 150%; nongenital, 117%) with 100% specificity compared with cell culture methods. Importantly, of specimens that may be submitted from victims of possible sexual abuse or from infants with pneumonia, 9 of 11 (vagina, 6; nares, 1; throat, 1; eye, 1) were exclusively detected and verified as positive by NAAT followed by direct fluorescent-antibody assay (DFA) confirmation. Interestingly, three of the specimens came from children with ages of 14 days (eye), 21 days (throat), and 33 days (nares).

The experimental design of our study was based on testing all specimens submitted in M5 medium for cell culture cultivation of *C. trachomatis*. This specimen was also suitable for nucleic acid extraction of *C. trachomatis* DNA that was amplified by the BDProbeTec ET NAAT. Evidenced-based information suggests that FDA-approved NAAT should be the preferred assay for the laboratory diagnosis of all *C. trachomatis* infections from all patient populations. All positive NAAT for *C. trachomatis* obtained from children should be verified by another FDA-approved method (such as DFA, enzyme immunoassay, or NAAT) to ensure specificity of the results (7, 9, 14, 17).

REFERENCES

1. **Becton Dickinson and Co.** 2003. BD ProbeTec TM ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* amplified DNA assay. Package insert. Becton Dickinson and Co., Sparks, Md.
2. **Boyadzhyan, B., T. Yashina, J. H. Yatabe, M. Patnaik, and C. S. Hill.** 2004. Comparison of the APTIMA CT and GC assays with the APTIMA Combo 2 assay, the Abbott LCx assay, and direct fluorescent-antibody and culture assays for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. J. Clin. Microbiol. **42:**3089–3093.
3. **Cheng, H., M. Macaluso, S. H. Vermund, and E. W. Hook III.** 2001. Relative accuracy of nucleic acid amplification tests and culture in detecting *Chlamydia* in asymptomatic men. J. Clin. Microbiol. **39:**3927–3937.
4. **de Barbeyrac, B., I. Pellet, B. Dutilh, C. Bebear, B. Dumon, and M. Geniaux.** 1994. Evaluation of the Amplicor Chlamydia trachomatis test versus culture in genital samples in various prevalence populations. Genitourin Med. **70:** 162–166.
5. **Gann, P. H., J. E. Herrmann, L. Candib, and R. W. Hudson.** 1990. Accuracy of *Chlamydia trachomatis* antigen detection methods in a low-prevalence population in a primary care setting. J. Clin. Microbiol. **28:**1580–1585.
6. **Gaydos, C. A., M. R. Howell, T. C. Quinn, J. C. Gaydos, and K. T. McKee, Jr.** 1998. Use of ligase chain reaction with urine versus cervical culture for detection of *Chlamydia trachomatis* in an asymptomatic military population of pregnant and nonpregnant females attending Papanicolaou smear clinics. J. Clin. Microbiol. **36:**1300–1304.
7. **Johnson, R. E., W. J. Newhall, J. R. Papp, J. S. Knapp, C. M. Black, T. L. Gift, R. Steece, L. E. Markowitz, O. J. Devine, C. M. Walsh, S. Wang, D. C. Gunter, K. L. Irwin, S. DeLisle, and S. M. Berman.** 2002. Screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections–2002. Morb. Mortal. Wkly. Rep. Recomm. Rep. **51:**1–38.
8. **Mahony, J. B., B. K. Coombes, and M. A. Chernesky.** 2003. Chlamydia and *Chlamydophila*, p. 991–1004. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, R. H. Yolken, and J. H. Jorgensen (ed.), Manual of clinical microbiology, 8th ed., vol. 1. ASM Press, Washington, D.C.
9. **Martin, D. H., M. Nsuami, J. Schachter, E. W. Hook III, D. Ferrero, T. C. Quinn, and C. Gaydos.** 2004. Use of multiple nucleic acid amplification tests to define the infected-patient "gold standard" in clinical trials of new diagnostic tests for *Chlamydia trachomatis* infections. J. Clin. Microbiol. **42:**4749–4758.
10. **Morre, S. A., I. G. van Valkengoed, A. de Jong, A. J. Boeke, J. T. van Eijk, C. J. Meijer, and A. J. van den Brule.** 1999. Mailed, home-obtained urine specimens: a reliable screening approach for detecting asymptomatic *Chlamydia trachomatis* infections. J. Clin. Microbiol. **37:**976–980.
11. **Page-Shafer, K., A. Graves, C. Kent, J. E. Balls, V. M. Zapitz, and J. D. Klausner.** 2002. Increased sensitivity of DNA amplification testing for the detection of pharyngeal gonorrhea in men who have sex with men. Clin. Infect. Dis. **34:**173–176.
12. **Schachter, J., W. M. McCormack, M. A. Chernesky, D. H. Martin, B. Van Der Pol, P. A. Rice, E. W. Hook III, W. E. Stamm, T. C. Quinn, and J. M. Chow.** 2003. Vaginal swabs are appropriate specimens for diagnosis of genital tract infection with *Chlamydia trachomatis*. J. Clin. Microbiol. **41:**3784–3789.
13. **Schwebke, J. R., W. E. Stamm, and H. H. Handsfield.** 1990. Use of sequential enzyme immunoassay and direct fluorescent antibody tests for detection of *Chlamydia trachomatis* infections in women. J. Clin. Microbiol. **28:**2473–2476.
14. **Taylor-Robinson, D.** 1996. Tests for infection with Chlamydia trachomatis. Int J. STD AIDS **7:**19–26.
15. **Taylor-Robinson, D., and B. J. Thomas.** 1991. Laboratory techniques for the diagnosis of chlamydial infections. Genitourin. Med. **67:**256–266.
16. **Van Der Pol, B., D. V. Ferrero, L. Buck-Barrington, E. Hook, 3rd, C. Lenderman, T. Quinn, C. A. Gaydos, J. Lovchik, J. Schachter, J. Moncada, G. Hall, M. J. Tuohy, and R. B. Jones.** 2001. Multicenter evaluation of the BDProbeTec ET System for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, female endocervical swabs, and male urethral swabs. J. Clin. Microbiol. **39:**1008–1016.
17. **Watson, E. J., A. Templeton, I. Russell, J. Paavonen, P. A. Mardh, A. Stary, and B. S. Pederson.** 2002. The accuracy and efficacy of screening tests for Chlamydia trachomatis: a systematic review. J. Med. Microbiol. **51:**1021–1031.
18. **Wilson, D. J., T. F. Smith, and D. M. Ilstrup.** 1984. Comparison of iodine- and fluorescein-labeled monoclonal antibodies for detection of Chlamydia trachomatis inclusions in cells grown in glass vials. Diagn. Microbiol. Infect. Dis. **2:**17–22.
19. **Workowski, K., and W. C. Levine.** 2002. Sexually transmitted diseases treatment guidelines—2002. Morb. Mortal. Wkly. Rep. Recomm. Rep. **51:**1–80.